

CLAIMS

1. A secreted polypeptide which has protease activity, which polypeptide comprises at least three non-polar or uncharged polar amino acids within the last four amino acids of the C-terminus of the polypeptide, and which polypeptide:

- 5 (a) comprises an amino acid sequence which is at least 70% identical to the amino acid sequence of the mature part of the polypeptide shown in SEQ ID NO: 28; SEQ ID NO: 33; SEQ ID NO: 37; SEQ ID NO: 41; SEQ ID NO: 43; or SEQ ID NO: 45;
- 10 (b) comprises an amino acid sequence which is at least 70% identical to the amino acid sequence of the mature part of the polypeptide encoded by the polynucleotide in SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 25; SEQ ID NO: 31; SEQ ID NO: 32; SEQ ID NO: 36; SEQ ID NO: 40; or SEQ ID NO: 44;
- 15 (c) comprises a mature part which is a variant of the mature part of the polypeptide having the amino acid sequence of SEQ ID NO: 28; SEQ ID NO: 33; SEQ ID NO: 37; SEQ ID NO: 41; SEQ ID NO: 43; or SEQ ID NO: 45 comprising a substitution, deletion, extension, and/or insertion of one or more amino acids;
- (d) is an allelic variant of (a), (b), or (c); or
- (e) is a fragment of (a), (b), (c), or (d).

2. The polypeptide according to claim 1, which is a wildtype polypeptide, an artificial variant of
20 a wildtype polypeptide said variant having one or more amino-acid(s) added to the C-terminus as compared to the wildtype, a shuffled polypeptide, or a protein-engineered polypeptide.

25 3. The polypeptide according to claim 2, wherein the one or more added amino acid(s) is (are) non-polar or uncharged.

4. The polypeptide according to claim 3, wherein the one or more added amino acid(s) is one or more of Q, S, V, A, or P.

30 5. The polypeptide according to claim 2, wherein the one or more added amino acids are selected from the group consisting of: QSHVQSAP, QSAP, QP, TL, TT, QL, TP, LP, TI, IQ, QP, PI, LT, TQ, IT, QQ, and PQ.

35 6. The polypeptide according to any of claims 1 – 5 which when expressed and before maturation comprises a heterologous pro-region from a different protease; preferably the pro-region is derived from an S2A or S1E protease; more preferably the pro-region is an artificial or shuffled pro-region, and most preferably it is at least 70% identical to the pro-region shown in

SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 33, SEQ ID NO: 37, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, or SEQ ID NO: 53.

- 5 7. The polypeptide according to any of claims 1 – 6 which when expressed and before maturation comprises a heterologous secretion signal-peptide which is cleaved from the polypeptide when the polypeptide is secreted, preferably the heterologous secretion signal peptide is derived from a heterologous protease.
- 10 8. The polypeptide according to claim 7, wherein the heterologous secretion signal peptide comprises an amino acid sequence having a sequence identity of at least 70% with the amino acid sequence encoded by polynucleotides 1 – 81 of SEQ ID NO: 2, or SEQ ID NO: 44.
- 15 9. An isolated polynucleotide encoding a polypeptide as defined in any of claims 1-8.
10. A recombinant expression vector or polynucleotide construct comprising a polynucleotide as defined in claim 9.
- 20 11. A recombinant host cell comprising a polynucleotide as defined in claim 9, or an expression vector or polynucleotide construct as defined in claim 10.
12. The recombinant host cell according to claim 11 which is a *Bacillus* cell.
- 25 13. A transgenic plant, or plant part, comprising a polynucleotide as defined in claim 9, or an expression vector or polynucleotide construct as defined in claim 10.
- 30 14. A transgenic, non-human animal, or products, or elements thereof, comprising a polynucleotide as defined in claim 9, or an expression vector or polynucleotide construct as defined in claim 10.
- 35 15. A method for producing a polypeptide as defined in any of claims 1 - 8, the method comprising: (a) cultivating a recombinant host cell as defined in claim 11 or 12, or a transgenic plant or animal as defined in claims 13 or 14, to produce a supernatant comprising the polypeptide, and optionally (b) recovering the polypeptide.

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16. An animal feed additive comprising at least one polypeptide as defined in any of claims 1 - 8; and

- (a) at least one fat-soluble vitamin, and/or
- (b) at least one water-soluble vitamin, and/or
- 5 (c) at least one trace mineral.

17. An animal feed composition having a crude protein content of 50 to 800 g/kg and comprising at least one polypeptide as defined in any of claims 1 - 8, or at least one feed additive of claim 16.

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18. A composition comprising at least one polypeptide as defined in any of claims 1 - 8, together with at least one other enzyme selected from amongst phytase (EC 3.1.3.8 or 3.1.3.26); xylanase (EC 3.2.1.8); galactanase (EC 3.2.1.89); alpha-galactosidase (EC 3.2.1.22); protease (EC 3.4.-.-), phospholipase A1 (EC 3.1.1.32); phospholipase A2 (EC 15 3.1.1.4); lysophospholipase (EC 3.1.1.5); phospholipase C (3.1.4.3); phospholipase D (EC 3.1.4.4); and/or beta-glucanase (EC 3.2.1.4 or EC 3.2.1.6).

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19. A method for using at least one polypeptide as defined in any of claims 1 - 8, for improving the nutritional value of an animal feed, for increasing digestible and/or soluble protein in animal diets, for increasing the degree of hydrolysis of proteins in animal diets, and/or for the treatment of vegetable proteins, the method comprising including the polypeptide(s) in animal feed, and/or in a composition for use in animal feed.

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20. A method for using at least one polypeptide as defined in any of claims 1 - 8, comprising including the polypeptide(s) in a detergent formulation.

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